

II. "Contributions to the Chemistry of Chlorophyll. No. VI."
By EDWARD SCHUNCK, F.R.S., and LEON MARCHLEWSKI,
Ph.D. Received January 25, 1895.

The preceding memoir on the chemistry of chlorophyll contained the results arrived at in the analysis of phyllotaonin and its derivatives. In the present communication it is proposed to give further details regarding the properties and composition of alkachlorophyll, the mother substance of phyllotaonin, on which will follow an account of some products of decomposition of the latter. Some experiments will then be described showing the connection between phylloxanthin and phyllocyanin, and proving that the former may by a simple process be converted into the latter.

In order to determine the composition of alkachlorophyll, it was necessary to prepare specimens of the substance purer than those previously obtained, which were only just sufficiently pure for a determination of its chief properties. For this purpose we employed, as in previous experiments, an extract of grass with boiling alcohol.

We also made use of a commercial product furnished by Merck of Darmstadt, under the name of "Chlorophyllum purissimum," which, on examination, turned out to be not exactly what its name would betoken, but a very impure alkachlorophyll.

In the case of grass, the first part of the process was the same as that described in the fourth memoir of this series, and the details may therefore be omitted here. The sodium salt of alkachlorophyll of this process was treated for some time with a mixture of equal parts of absolute alcohol and ether, in order to remove the fatty acid soaps present. The sodium salt was then decomposed in the manner previously described, and the free alkachlorophyll obtained having been dissolved in ether, the solution was mixed with ligroin.

The latter precipitated the alkachlorophyll, the fatty acids still present remaining dissolved. The solution in ether and precipitation with ligroin was twice repeated, and the resulting product was treated several times with small quantities of boiling ligroin.

The product supplied by Merck, after being treated for some time with alcoholic soda, was submitted to the same process as that just described.

The substance dried at 125° yielded on analysis the following results, the first two determinations being made with the product from grass, the third with that from Merck's "Chlorophyll."

I. 0.1875 gram substance gave 0.4813 gram CO₂ and 0.1101 gram H₂O.

0·1968 gram substance gave 19·7 c.c. of nitrogen at 23° and 745 mm. pressure.

II. 0·1744 gram substance gave 0·4478 gram CO₂ and 0·0974 gram H₂O.

0·2293 gram substance gave 23·3 c.c. of nitrogen at 24° and 764 mm. pressure.

III. 0·2145 gram substance gave 0·5500 gram CO₂ and 0·1242 gram H₂O.

0·1072 gram substance gave 10·70 c.c. of nitrogen at 21° and 757 mm. pressure.

These numbers correspond in 100 parts to—

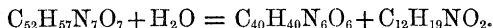
	I.	II.	III.	Mean.
C	70·00	70·00	69·93	69·97
H	6·52	6·20	6·43	6·37
N	11·03	11·43	11·31	11·27

There are two formulæ with which these numbers agree equally well, viz., C₃₀H₃₄N₂O₄ and C₅₂H₅₇N₇O₇, requiring respectively

	C ₃₀ H ₃₄ N ₂ O ₄ .	C ₅₂ H ₅₇ N ₇ O ₇ .
C	70·04	70·12
H	6·61	6·39
N	10·89	10·99
O	12·46	12·50

Since phyllotaonin, with the formula C₄₀H₄₆N₆O₆, is formed by the action of acids on alkachlorophyll, it was necessary to adopt for the latter a formula with no less than 6 atoms of nitrogen. The second of the above formulæ meets this requirement.

The formation of phyllotaonin from alkachlorophyll by the action of acids, supposing the process to be one of simple hydrolysis, might be represented by the following equation:—



That a small quantity of a product of a basic nature probably containing nitrogen is formed along with phyllotaonin, by the action of acids on alkachlorophyll, has already been mentioned in the fourth memoir of this series, but we have not been able to throw any more light on the nature of this product. That some substance containing nitrogen must be formed together with phyllotaonin in the decomposition, is also indicated by the equation just given, but whether the formula C₁₂H₁₉NO₂ represents one substance only, or a mixture of several, is doubtful.

The properties of alkachlorophyll have been already described, and need not therefore be referred to here. The action of alkalis on it at a high temperature may however be briefly described.

When a solution of alkachlorophyll in alcoholic potash is heated in a sealed tube for several hours at 190—200° it is decomposed; on opening the tube no pressure is perceived.

The contents, consisting partly of small brownish-red crystals, having been poured out, an excess of hydrochloric acid is added; this dissolves a part, yielding a purple solution.

The latter is filtered off from the undissolved brown mass, and after being nearly neutralised with soda, yields on agitation with ether a solution of a fine crimson colour. The ethereal solution after washing with water leaves on evaporation a quantity of small crystals. The substance so obtained is identical with the one formed by the same process from phyllotaonin and which will be described presently. It seems also to be essentially the same as that obtained by Tschirch in acting on alkachlorophyll with alkali, and called by him "phylloporphyrin acid," but a comparison of the absorption spectrum of the latter as described by him with that of our substance shows that his product must have been impure. This is evident from the fact that the absorption spectrum of Tschirch's product shows, according to him, a band in the red about in the same position as band I of the chlorophyll spectrum, whereas this band though visible in solutions of our substance when the latter is not quite pure, disappears entirely in solutions of the pure substance.

The liquid filtered off from the products insoluble in hydrochloric acid of the process just described, contains in combination with the acid various volatile bases, chiefly ammonia. In one experiment the liquid, after being made alkaline with soda, was submitted to distillation, the vapour evolved being passed into dilute hydrochloric acid. The acid liquid after concentration yielded with platinic chloride a yellow precipitate, which was collected, treated with boiling alcohol, and dried.

0.1031 gram of the substance gave 0.0451 gram platinum = 43.74 per cent.; calculation for $(\text{NH}_4)_2\text{PtCl}_6$ requires 43.90 per cent.

The filtrate from the platinum ammonium chloride yielded, on evaporation, orange coloured octahedra, containing 38.7 per cent. of platinum. It may hence be inferred that along with ammonia some base of higher molecular weight is formed; the quantity is, however, very small.

Phylloporphyrin.

The action of caustic alkalis on chlorophyll and its derivatives has been previously investigated. Hoppe-Seyler obtained by acting on his chlorophyllan with alkali at 260—290° a substance giving purple solutions. On account of the double fluorescence of its solutions observed by him he gave it the name of "dichromatic acid." The discoverer states that it contains no nitrogen, the analysis of the

barium salt leading for the acid to the formula $C_{20}H_{34}O_3$, and it is, he states, a very unstable body. The absorption spectrum of its solutions shows six bands in the relative positions indicated by him. Among the products of decomposition of dichromatic acid mention is made of a substance which in acid solutions shows only two bands; this substance Hoppe-Seyler calls phylloporphyrin. Another product of decomposition is obtained, according to him, on evaporating the ethereal solution. Sachsse obtained by fusion of his β -phæochlorophyll with soda a reddish-brown product dissolving in acids with a violet colour, and having the formula $C_{26}H_{33}N_3O_2$. Similar experiments made with phyllocyanin, which led to the formation of a purple crystallised substance, have already been described in the fourth memoir of this series. The product formed in all these cases being doubtless the same, we shall adopt for it the name phylloporphyrin, Hoppe-Seyler's body of the same name being, in our opinion, simply a compound with acid, not a product of decomposition. The substance has, in fact, a pronounced basic character, and shows in neutral solutions a spectrum differing entirely from that of its acid solutions.

Preparation and Properties of Phylloporphyrin.

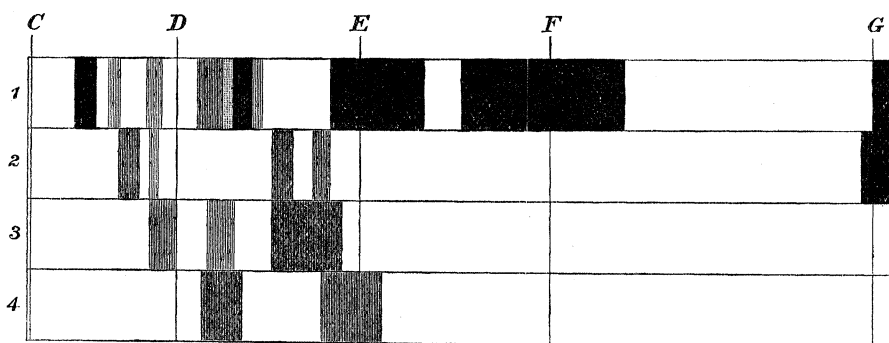
The method of preparing the substance from phyllocyanin has previously been described. The method before given is, however, not an advantageous one, the yield being trifling, and the quantity of bye-products formed at the same time relatively large. We therefore adopted another process, avoiding the use of melted alkali in open vessels and employing alcoholic potash in sealed tubes. The material employed was phyllotaonin, which, being itself a product of decomposition of phyllocyanin with alkali at lower temperatures, and being more easily prepared in a state of purity than phyllocyanin, seemed better adapted than the latter for the preparation of phylloporphyrin. The process employed was as follows:—Ethyl phyllotaonin in crystals was treated with alcoholic potash in sealed tubes at 190° for several hours. On opening the tubes no pressure was observed. The brownish-red contents were poured out, mixed with water and an excess of hydrochloric acid, and then shaken up with ether. The ether acquired a fine purple colour, and left on evaporation a brownish residue in which dark purple crystals were discernible. The residue was treated with boiling alcohol, which dissolved part, leaving a quantity of brown matter undissolved. On adding to the filtrate an alcoholic solution of zinc acetate, and allowing it to stand, a red crystalline deposit—a zinc compound of phylloporphyrin—was formed. This was collected and dissolved in boiling alcohol. The solution, after addition of a little hydrochloric acid, was mixed

with much water and extracted with ether. The latter acquired a splendid crimson colour, and, having been washed with water, left on evaporation reddish-violet crystals, which were recrystallised from alcohol.

As thus prepared, phylloporphyrin appears in the form of lustrous crystals of a splendid reddish-violet colour, which under the microscope are seen to be short regular prisms. It is tolerably soluble in alcohol and ether, giving red fluorescent solutions, which, on the addition of a little acid, acquire a bluish tint. It dissolves in mineral acids as well as in glacial acetic acid. The solutions give up nothing when shaken up with ether, even when previously mixed with much water, showing that they contain compounds of the substance with the respective acids. When, however, the solutions are made alkaline and then again slightly acid, then ether, on agitation, takes up the substance. On adding nitric acid to the solution in acetic acid it becomes greenish, and, on heating, the colour disappears entirely. Phylloporphyrin possesses also acid properties, though these are not very pronounced. It is, indeed, quite insoluble in aqueous alkalis; but when caustic soda is added to its alcoholic solution a brown sodium compound separates, which yields up nothing to ether until the alkali has been removed by means of acetic acid. It yields, moreover, as before mentioned, a crystallised zinc salt, which is formed on adding zinc acetate to its alcoholic solution, when it crystallises out in small red needles; the compound is decomposed by hydrochloric acid, but it is scarcely changed by acetic acid. The zinc salt is of some importance in connection with the preparation of the pure substance, since by its means it becomes possible to separate it from other products of decomposition, more especially from the brown amorphous substances accompanying it.

As regards its absorption spectrum, phylloporphyrin is a very interesting substance. The ethereal solution shows seven bands, which are, for the most part, very sharply defined (see fig.); it is the first of the derivatives of chlorophyll, so far discovered, which shows in solution no band within the red, the first band being at the margin of and almost outside that part of the spectrum. The spectrum of the alcoholic solution is similar, but the second and third bands are not seen, and the fourth and fifth bands appear as one. The alcoholic solution to which hydrochloric acid has been added shows an absorption spectrum of three bands only, one close to line B, another near E, and a third, which is very faint, between the two. This spectrum should, we thought, have been the same as that of Hoppe-Seyler's phylloporphyrin, which we consider to be a compound of our phylloporphyrin with acid; that they are not quite identical is probably due to Hoppe-Seyler's product having been impure. That a solution of phylloporphyrin to which hydrochloric acid has been

added really contains a compound with acid and not a product of decomposition, is evident from the fact that if the acid be neutralised with soda the solution loses its bluish tinge, and now shows the absorption spectrum of an alcoholic solution of the free substance. A solution of phylloporphyrin in concentrated sulphuric acid shows a spectrum with four bands. The spectrum of solutions of the zinc salt is peculiar. While the solutions of the alkaline salts show a spectrum differing very little from that of the substance itself, the zinc salt in solution shows only two bands (see fig.). On adding a little hydrochloric acid to the zinc salt solution and boiling, the spectrum of the hydrochloride makes its appearance, followed, after considerable dilution, by that of the substance itself.



EXPLANATION OF FIGURE.

Absorption Spectra of Phylloporphyrin and its Compounds.

1. Phylloporphyrin in ether.
2. The same in concentrated sulphuric acid.
3. The same in hydrochloric acid.
4. Zinc salt.

Phylloporphyrin, like most of the derivatives of chlorophyll hitherto examined, contains nitrogen, differing in this respect from the dichromatic acid of Hoppe-Seyler, which, according to him, is devoid of nitrogen.

The analysis of phylloporphyrin and of its zinc salt yielded the following results:—

0.1023 gram phylloporphyrin gave 0.2850 gram CO_2 and 0.0654 gram H_2O .

0.0920 gram gave 9.00 c.c. of nitrogen at 21° and 753 mm. pressure.

0.0848 gram of the zinc salt gave 0.2093 gram CO_2 and 0.0466 gram H_2O .

These numbers correspond in 100 parts to—

	Phylloporphyrin.	Zinc salt.
C	75·98	67·31
H	7·10	6·10
N	11·02	—

The formulæ $C_{32}H_{34}N_4O_2$ and $C_{32}H_{32}N_4O_2Zn$ require respectively—

	Phylloporphyrin.	Zinc salt.
C	75·89	67·46
H	6·73	5·63
N	11·06	—
O	6·32	—

Want of substance prevented our making further determinations in confirmation of those above given.

It is difficult at present to explain in a simple manner the formation of phylloporphyrin from phyllotaonin, the more so since phylloporphyrin is not the only product of decomposition due to the action of alkali, it being always accompanied by a relatively large quantity of brown amorphous substances as before mentioned. Ammonia, together with a small quantity of another base having a higher molecular weight, could also be detected in the acid liquid filtered off from the phylloporphyrin and the brown substances, just as in the case of alkachlorophyll; the presence of these bodies was discovered in the manner previously described.

Conversion of Phylloxanthin into Phyllocyanin.

Phylloxanthin and phyllocyanin being both products of decomposition of chlorophyll with acids, and having many properties in common, it seemed probable that a connection of an intimate character might be found to subsist between the two substances. Our experiments lead to the conclusion that this is, in fact, the case, and that phylloxanthin may by the prolonged action of acids be converted into phyllocyanin. This convertibility has frequently been suspected, but has never yet been satisfactorily proved.

The changes which take place in the spectrum of chlorophyll on the addition of acids to its solutions have long been known, they have been accurately described by Russell and Lapraik in their memoir entitled “A Spectroscopic Study of Chlorophyll.”*

According to these observers, when a few drops of hydrochloric acid are added to an ethereal solution of chlorophyll, the colour of the latter changes from bright green to yellowish-green. At the same time the spectrum of the solution undergoes a marked change: bands

* ‘Chem. Soc. Trans.,’ vol. 41, p. 334, 1882.

I and II appear more clearly defined in consequence of the clearing up of the space between them, while band III is shifted nearer the blue end and becomes paler, band IV, on the other hand, becoming much darker. After the solution has stood for a few days a fifth band nearer the blue end, and equal in intensity to the fourth, appears. The same changes take place, but more slowly, when tartaric acid is employed in place of hydrochloric acid. When acetic acid, however, is taken, the solution remains unchanged for some time; it then becomes yellowish-green and shows four bands as before, but the fifth band never makes its appearance however much the action may be prolonged. These changes in the spectrum of chlorophyll solutions on the addition of various acids may be explained by supposing that phylloxanthin is formed in the first instance, and is then, by the continued action of the acid, converted into phyllocyanin. The conversion though slow, on the whole proceeds more rapidly at the commencement than towards the end of the process, and is evidently due to hydrolysis. Similar phenomena are observed in the case of melitose, the metamorphosis of which also passes through two stages, being first converted by the action of emulsin into melibiose, which then by hydrolysis with acid yields galactose and fructose.

The phylloxanthin employed in the experiments about to be described was obtained in accordance with the directions given in the fourth memoir of this series. We have nothing to add to the account there given of the properties of phylloxanthin, except that when quite pure its solutions show only four absorption bands, not five as there stated, a fifth band, when it appears, indicating the presence of phyllocyanin.

Into an ethereal solution of phylloxanthin free from phyllocyanin, a current of dry hydrochloric acid gas was passed, the solution being kept cool, until quite saturated, and it was then shaken up with concentrated hydrochloric acid. The latter gradually acquired a bluish-green colour, which became darker on standing. The two liquids having remained in contact for some time were separated, and the lower acid one was mixed with several times its volume of water, which gave an almost black flocculent precipitate. The whole was then shaken up with ether, in which the precipitate dissolved, yielding a solution which, after washing with water, showed the olive-green colour peculiar to solutions of phyllocyanin as well as the five absorption bands belonging to the latter in their usual position and with their usual relative intensities. That a formation of phyllocyanin had actually taken place was proved by the following experiments. The ethereal solution of the product obtained was evaporated. Part of the residue left was dissolved in caustic soda; the solution, after standing for some time, was supersaturated with acetic acid, and then shaken up with ether; the ethereal solution was evaporated,

and the residue was dissolved in boiling glacial acetic acid; the solution on standing deposited small crystalline grains, the ethereal solution of which showed the absorption spectrum of acetylphyllotaonin. Another portion of the residue left on evaporation of the ethereal solution was dissolved in boiling alcoholic potash; the solution, after filtration and addition of water, was acidified with acetic acid and then shaken up with ether; an ethereal solution was obtained showing the characteristic absorption spectrum of phyllotaonin.

The experiments just described proving the convertibility of phyloxanthin into phyllocyanin, tend to support the view held by some observers that only one chlorophyll really exists, and that there are not two or more chlorophylls as has sometimes been maintained. Under the action of acids, chlorophyll yields phyloxanthin, which is then converted into phyllocyanin. The latter when treated with alkalis gives phyllotaonin, and this, by the more energetic action of alkalis at a higher temperature, yields phylloporphyrin. The progressive increase in the number of absorption bands in passing through this series, and the gradual shifting of the bands from the red to the blue end of the spectrum, are points worthy of notice.

III. "On the Ratio of the Specific Heats of some Compound Gases." By J. W. CAPSTICK, D.Sc., M.A., Fellow of Trinity College, Cambridge. Communicated by Professor J. J. THOMSON, F.R.S. Received January 25, 1895.

(Abstract.)

The experiments described are a continuation of those of which an account was given in the 'Philosophical Transactions,' vol. 185, p. 1.

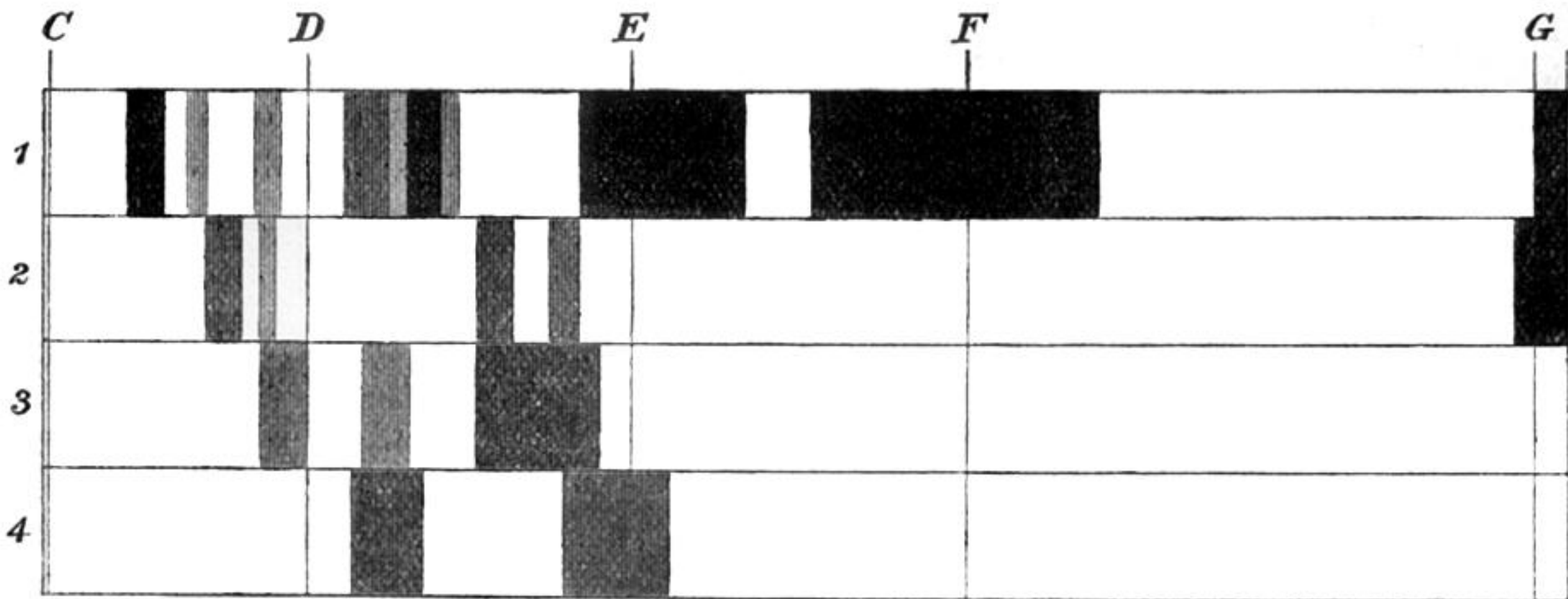
The apparatus and method of procedure were the same as were described in the former paper. Kundt's dust-figure method was used for finding the velocity of sound in the gas, and the ratio of the specific heats was calculated by means of the formula

$$\gamma = 1.408 \times \rho \times \left(\frac{l}{l'} \right) \left\{ 1 + \frac{1}{p} \frac{d}{dv} (pv) \right\},$$

the last factor being added to the ordinary formula in order to correct for the deviation of the gas from Boyle's law.

The densities and isothermal curves were determined experimentally.

The results are as follows :—



EXPLANATION OF FIGURE.

Absorption Spectra of Phylloporphyrin and its Compounds.

1. Phylloporphyrin in ether.
2. The same in concentrated sulphuric acid.
3. The same in hydrochloric acid.
4. Zinc salt.